

AMENDMENT TO THE SPECIFICATION

Please amend the specification as follows:

At page 1, first full paragraph:

This application is a continuation-in-part of U.S. Serial No. 09/271,777, now U.S. Patent No. 6,162,643, issued December 19 2000, entitled PROCESS AND MEDIA FOR THE GROWTH OF HUMAN EPITHELIA; which is a continuation of U.S. Serial No. 09/133,386, filed August 13, 1998, now U.S. Patent No. 5,912,175; which is a continuation of U.S. Serial No. 08/893,195, filed July 15, 1997, now U.S. Patent 5,834,312 issued November 10, 1998, entitled PROCESS AND MEDIA FOR THE GROWTH OF HUMAN EPITHELIA; which is a continuation-in-part of U.S. Serial No. 08/500,744, filed July 11, 1995, now U.S. Patent 5,686,307 issued November 11, 1997, entitled SERUM-FREE MEDIUM FOR USE IN THE FORMATION OF A HISTOLOGICALLY COMPLETE LIVING HUMAN SKIN SUBSTITUTE; which is a continuation of U.S. Serial No. 08/318,221, filed October 5, 1994, now abandoned; which is a continuation of U.S. Serial No. 08/184,905, filed January 21, 1994, now abandoned; which is a continuation of Serial No. 08/063,247, filed May 18, 1993, now abandoned; which is a divisional of U.S. Serial No. 07/471,976, filed January 29, 1990, now U.S. Patent No. 5,292,655. U.S. Patents 5,292,655, 5,686,307, 5,834,312, 5,912,175, and 6,612,643 are incorporated herein by reference.

At page 16, paragraph 3:

Figures 1-3 [[1B-D]] are phase contrast microscope photomicrographs of living cultures of keratinocytes cultured for 72 hours either in the presence of complete HECK-109 FS medium (B), standard HECK 110 medium (C) or standard HECK 109 medium (D).

At page 16, paragraph 4:

Figure 4 [[2A]] is a photograph of living cultures of keratinocytes that have been cultured for 1 hour and stained with crystal violet ~~violet~~ (0.2%). Figures 5-7 [[2B-D]] are photographs of the results of the same experiment in which the cultures were fixed at 72 hours after the above treatments and stained with crystal violet ~~violet~~ (0.2%).

At page 16, paragraph 5:

Figure 8 [[3A]] is a photomicrograph of a phase contrast microscope image of a living epidermal sheet produced by monolayer culture of keratinocytes in the inventive protein-free defined HECK-110 medium, and refed upon reaching confluency ~~confluency~~ with standard HECK medium containing 10% FBS and 1mM Ca²⁺ ions. Figure 9 [[3B]] is a photomicrograph of a living sheet of epidermis released from the plastic substrate of the culture dish by Dispase proteolytic.

At page 17, paragraph 1:

Figures 10-11 [[4A-B]] are photomicrographs of keratinocyte cultures cultured in complete HECK-109 FS medium and treated in an identical manner with 10% FBS and 1mM Ca²⁺ ions to yield a stratified epidermal epithelium.

At page 26, second full paragraph:

Human keratinocyte cultures were initiated from neonatal foreskin as described in Example 1, and then placed in secondary culture in complete HECK-109 FS medium. The purpose of the following experiment was to determine the effect of retinyl acetate on the proliferation of keratinocyte cultures refed HECK-109 basal medium lacking EGF

and IGF-1 and supplemented with hydrocortisone, ethanolamine, and phosphoethanolamine. For this purpose, duplicate secondary cultures were refed either 1) complete HECK-109 FS medium (positive control) containing 0.1 mM Ca^{2+} , 2) standard HECK-109 medium, i.e., basal medium supplemented with only hydrocortisone, ethanolamine, and phosphoethanolamine, and containing 0.1 mM Ca^{2+} and 3) standard HECK-109 supplemented with retinyl acetate ($3 \times 10^{-8}\text{M}$) (now called HECK-110). Figures 1-3 [[1B-D]] are phase contrast microscope photomicrographs of living cultures of keratinocytes cultured for 72 hours either in the presence of complete HECK-109 FS medium (B), standard HECK 110 medium (C) or standard HECK 109 medium (D). Two important observations were made. First, the colony morphology of retinyl acetate treated keratinocyte cultures displays a loose colony configuration, which is characteristic of proliferating cultures and the like that observed for the cultures maintained in growth factor replete medium. In addition, many dividing cells were observed both in the growth factor supplemented and in cultures refed growth factor-deficient medium supplemented with retinyl acetate. None were observed in cultures refed growth factor-deficient medium. The latter displayed a compact colony morphology characteristic of growth-arrested keratinocytes that have committed to terminal cell differentiation. This experiment has been repeated four times with the same results.

At page 27, first full paragraph:

Figure 4 [[2A]] is a photograph of living cultures of keratinocytes that have been cultured for 1 hour and stained with crystal violet ~~violet~~ (0.2%). Figures 5-7 [[2B-D]] are photographs of the results of the same experiment in which the cultures were fixed at 72 hours after the above treatments and stained with crystal violet ~~violet~~ (0.2%). The photographs show that cultures fed complete growth factor containing medium (B) had the most colonies, while cultures refed growth factor-deficient medium

supplemented with retinyl acetate (D) has many more colonies than cultures refed only growth-factor deficient standard medium (C).

At page 28, first full paragraph:

For this example, a viable living epidermal sheet of tissue of normal human keratinocytes was produced by first isolating keratinocytes by use of cell competency solution (CCS, U.S. Patent No. 5,795,281 to J. Wille), and then culturing the primary culture in complete keratinocyte growth medium (HECK-109 FS), as described in U.S. Patent No. 5,686,307 to J. Wille. Early passage cultures were further sub-cultivated in complete HECK-109 FS. Duplicate low cell density ($5 \times 10^3/\text{cm}^2$) culture refed protein-free defined HECK-110 medium for an additional 5 days until a confluent monolayer was formed. The cultures were then washed twice with HECK-109 standard media, i.e., basal medium containing ethanolamine (10^{-4} M, and phosphoethanolamine (10^{-4} M), and hydrocortisone (5×10^{-7} M), and refed HECK-110 standard medium containing 10% fetal bovine serum and 1mM Ca^{2+} ions for an additional two days. Figure 8 [[3A]] is a photomicrograph of a phase contrast microscope image of a living epidermal sheet produced by monolayer culture of keratinocytes in the inventive protein-free defined HECK-110 medium, and refed upon reaching confluence with standard HECK medium containing 10% FBS and 1mM Ca^{2+} ions. Figure 9 [[3B]] is a photomicrograph of a living sheet of epidermis released from the plastic substrate of the culture dish by Dispase proteolytic enzyme treatment (2mg/ml for 20 minutes). The general appearance of and morphology of the reformed human epidermis so formed was similar to duplicate keratinocyte cultures cultured in complete HECK-109 FS medium and treated in an identical manner with 10% FBS and 1mM Ca^{2+} ions to yield a stratified epidermal epithelium (Figures 10 [[4A]] and 11 [[4B]]).